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RESEARCH ARTICLE

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Mating behaviour and gamete release in gilthead seabream (*Sparus aurata*, Linnaeus 1758) held in captivity

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Abstract

The present study aimed to describe the reproductive behaviour of gilthead seabream (*Sparus aurata*) in captivity. Twenty-four mature gilthead seabream, divided in two tanks, were utilized for the present study. Reproductive behaviour was recorded using submersibles cameras. A total of 67 spawning events were analysed. The mean duration time that gilthead seabream spent spawning was 54 ± 4 min/day, during which mean number of individual spawning events was 5.6 ± 0.2. The mean volume of eggs produced by both broodstocks was 405 ± 13.4 mL with a fertilization rate of 91.6 ± 0.4%. The reproductive behaviour began with a schooling behaviour and then forming light aggregations. From an aggregation or an encounter while swimming freely a female initiated a spawning rush followed by one or more males to gametes liberation. The spawning rush was brief, 1.6 ± 0.5 sec, over an approximately 1.7 ± 0.2 m distance from the tank bottom to the water surface. Pair spawning, between a single female and male, was the most common (71.6%). Group spawning was less common and involved a single female spawning with two males (22.5%) or three males (4.9%). Spawning rushes involving more than one female were not observed. Gilthead seabream in the present study presented a tendency to pair spawn and eggs collected as a "spawn" were actually the sum of many separate spawning events over a short time period. This is the first description of gilthead seabream spawning and the findings help to understand microsatellite based observations of spawning kinetics.
Additional key words: mate selection; pair group mass spawning; rush; aggregation
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Introduction

Gilthead seabream (*Sparus aurata*), from the Sparidae family, is one of the most extensively farmed fish species in the Mediterranean region. During the last two decades, many studies have described aspects of the biology of the species, including reproduction and genetics (Holland *et al*., 1998; Almansa *et al*., 1999; Meiri *et al*., 2004; Rossi *et al*., 2006; Arabaci *et al*., 2010; Mylonas *et al*., 2011). Gilthead seabream is a protandrous hermaphrodite species with an asynchronous ovarian development (Zohar *et al*., 1995). Broodstock held in captivity under natural conditions typically start vitellogenesis in September-November, spawning begins during December-January and lasts for 3-5 months with daily spawning, leading to an annual fecundity of 2,000,000 eggs/kg (diameter<1 mm) with a fertilization rate of 80-85% (Barbaro *et al*., 1997; Arabaci *et al*., 2010; Mylonas *et al*., 2011). However, the reproductive behaviour of gilthead seabream has not been reported, despite an increasing need to understand the factors that influence a breeders participation in spawning in order to control the families produced from a broodstock (Gorshkov *et al*., 1997; Brown *et al*., 2005; Porta *et al*., 2009; Chavanne *et al*., 2012).

Reproductive behaviour has been described in some species of the family Sparidae, kept in captivity, including silver seabream (*Chrysophrys auratus*) (Smith, 1986; Mylonas *et al*., 2011), santer seabream (*Cheime-*

rius nufar) (Buxton & Garratt, 1990; Garratt, 1991), roman seabream (*Chrysoblephus laticeps*) (Buxton, 1990), silver bream (*Rhabdosargus sarba*) (Leu, 1994) and southern black bream (*Acanthopagrus butcheri*) (Mylonas *et al*., 2011). Although there was variation among species a general similarity was observed (see review in Mylonas *et al*., 2011). Spawning was usually early morning (dawn) or early evening (dusk) (06:00 and 19:00, respectively). Courtship consisted of males pursuing and nudging females, a tight circling swimming behaviour to form aggregations before spawning, which consisted of a spawning rush usually either to perform pair spawning involving a single pair (a male and a female) or group spawning a single female followed by multiple males (Smith, 1986; Buxton & Garratt, 1990; Garratt, 1991; Leu, 1994; Mylonas *et al*., 2011).

A number of studies have examined gilthead seabream parental contribution to spawning events as there is a need to genetically improve cultured gilthead seabream to obtain desirable traits such as faster growth that will reduce production costs (Gorshkov *et al*., 1997; Brown *et al*., 2005; Porta *et al*., 2009; Chavanne *et al*., 2012; Duncan *et al*., 2013). Gilthead seabream spawning success was low when held in pairs (22% success) or groups of 15 females with a single male (44% success) and gilthead seabream were difficult to strip spawn for artificial fertilisation (Gorshkov *et al*., 1997). Different authors have concluded that large groups of breeders are required for successful spawning of gilthead seabream (Gorshkov *et al*., 1997; Duncan *et al*., 2013) and Sparidae in general (Pankhurst, 1998; Mylonas *et al*., 2011). Parental assignment of progeny using microsatellites identified that although large broodstocks produce large volume spawns to which many breeders contributed the participation of breeders was variable and a proportion of breeders did not participate in spawning (Brown *et al*., 2005; Porta *et al*., 2009; Chavanne *et al*., 2012). Consequently, the effective spawning population size was reduced compared to the actual number of breeders in the broodstock, inbreeding was higher than expected and the families obtained were not predictable. Brown *et al*. (2005) and Chavanne *et al*. (2012) referred to gilthead seabream spawning behaviour as mass-spawning, which has been defined as "*spawning that consists of the great majority to all of an aggregation spawning simultaneously, as a single unit*" (Domeier & Colin, 1997).

Therefore, there is a need to study the spawning behaviour of gilthead seabream to increase the understanding of spawning in Sparidae and to enable geneticists and broodstock managers to understand the parental contributions obtained for genetic improvement programmes. The aim of the present study was to investigate and describe the particularities of reproductive behaviour of the gilthead seabream in rearing conditions.

Material and methods

Ethic statement

All the experimentation on fish that formed part of this study were in agreement with the Spanish and European regulations on animal welfare (Federation of Laboratory Animal Science Associations, FELASA) and approved by the Animal Ethics Committee of IRTA.

Fish maintenance

Twenty four mature gilthead seabream (*Sparus aurata*) with a mean weight of 2.59 ± 0.15 kg and a length of 49 ± 4 cm were used for this study. Fish were pit-tagged for identification and divided among two 16.2 m^3 rectangular (6 × 3 × 0.9 m) fibreglass tanks (identified ahead as C1 and C2). Sex ratio per tank was 7 females and 5 males; a ratio biased to females is commonly used in the industry. Females were larger and older (mean weight: 2.91 ± 0.12 kg) than males (mean weight: 2.27 ± 0.17 kg), and this morphological difference was established as the main criteria to distinguish males from females in the video recordings.

Tanks were located outside in a greenhouse structure covered with shade netting. Photoperiod was adjusted to follow the natural seasonal cycle by using two halogen white lights installed inside of each tank. Lights turned on-off in tanks with a photocell sensor. Water temperature and oxygen were maintained between 18-19°C and 5-6 mg/L, respectively. Fish were fed, *ad-libitum,* daily in the mornings (between 09:00-10:00 hours) with a commercial extruded balanced diet (Vitalis CAL-9, Skretting, Burgos, Spain).

Video and observations of the reproductive behaviour

Fish behaviour was recorded with four submersible black and white cameras (F60B/NIR580-50G model, Korea Technology Co. Ltd, supplied by Praentesis S.L., Barcelona) connected to a recorder (DVR- 0404HB model, Dahua Technology Co. Ltd, supplied by Praentesis S.L., Barcelona). Cameras were installed in each tank 5 cm under the water surface and adjusted to achieve a field of vision that covered more than 95% of the area and water column of the tanks.

The video recording was completed on different dates for both tanks as only one video recording system was available. Tank C1 behaviour was recorded from 10th to 24th January and from the 1st to the 4th February 2012; subsequently, tank C2 was recorded from the 5th to 14th February and from 30th of May to 7th of June 2012. The video recording program was daily starting at 08:00 until 13:00 hours in both tanks. This schedule was determined in relation to egg collection, generally collectors were observed to be empty at 08:00 hours and after collection at 13:00 hours no more eggs were collected until the following day after 08:00 hours.

Focal animal observations of spawning behaviour and behaviour in general were made from the recorded videos following recommendations published by Altman (1974). A total of 67 spawning events were analysed. In tank C1, spawning observations corresponded to days 12th, 13th, 16th and 18th January and 1st-2nd February, whilst in tank C2, observations corresponded to days 05th, 09th-12th February and 30th May. The following types of behaviours and observations were described from the videos: i) pre-spawning interactions between individuals or in a group, ii) the behaviour directly associated with gamete liberation, iii) fish aggregation patterns and duration, iv) number of fish participating in each spawn (pair or group spawning) and the sex proportion per spawn, v) the frequency, duration and position of fish in tank when spawning and vi) estimation of the average distance (estimated from known distances between reference points in the tank) of the spawning rush. These parameters were selected in accordance to previous work realized on Sparidae species (Smith, 1986; Buxton & Garratt, 1990; Garratt, 1991; Leu, 1994; Mylonas *et al*., 2011) and in particular terminology defined by Domeier & Colin (1997) was used to describe behaviours and actions. These included the following definitions of types of spawning from Domeier & Colin (1997) "*Pair spawning: spawning by a single male and single female. Group spawning: rush consisting of more than two fish, often many individuals. The group usually consists of a single female and multiple males. Mass spawning: a form of group spawning that consists of the great majority to all of an aggregation spawning simultaneously, as a single unit"*.

Eggs collection and evaluation

Egg collection was daily between 11:30 and 12:00 hours from both tanks. A 2-L measuring cylinder was used to measure the total volume of spawned eggs and the fertilization rate was determined by counting fertilized and unfertilized eggs from a sample of 50 eggs. Fertilized eggs were identified by observing cellular divisions, while unfertilized eggs did not present any cellular divisions. Likewise, the developmental stage of the embryonic phase of eggs was analyzed and established with accordance to Kamaci *et al*. (2005), in order to corroborate the estimates of spawning time obtained from videos with the developmental stage of eggs.

Statistics

All data were expressed in mean ± S.E.M. Student´s t- test was performed to compare different behavioural patterns between the two broodstocks (tank C1 and C2), such as the total number of aggregations prior a spawning, spawning duration, frequency of spawns per day, the distance displaced to spawn and the sex proportion per spawn. Pearson correlation test was performed between the number of daily events of gamete release and the volume of eggs collected. All the statistical analyses were conducted using SPSS software (Chicago, IL, USA) and a significant difference was considered when $p < 0.05$.

Results

Observations and description of the sea bream reproductive behaviour

Based on the video-observations, the gilthead seabream reproductive behaviour was divided into two phases: the pre-spawning and the spawning behaviour. It was noted that seabream in the present study had a tendency to spawn daily in both tanks with close to all eggs being spawned between 08:00 and 11:00 hours. However, a small number of spawns were outside of these hours.

Pre-spawning behaviour

— *Resting behaviour.* Resting behaviour was observed when lights in both tanks were turned on (on average activated by photocell sensor at 08:30 hours). This behaviour was characterized with fish totally dispersed, without interactions and disaggregated around the tanks, and fish swam alone or in small groups around the tank without any specific direction or preference (Fig. 1, Table 1).

— *Schooling behaviour.* On average 42 ± 8 min after the lights were turned on, fish behaviour changed and fish started to form groups and swim together following one behind another from one side of the tank to the other. However, fish did not present any specific direction or preference, but always were swimming near the bottom of the tank. It was also observed that some fish maintained a reduced distance in relation to other fish and this included some fish touching or sneaking after each other (Fig. 1, Table 1). This behaviour pattern was observed daily in both tanks for approximately 10 min and prior to the aggregation behaviour. However, no particular leading fish or inter-individual dominance between fish could be observed amongst the individuals of both tanks.

Spawning behaviour

— *Aggregations and courtship behaviour.* Aggregations and courtship behaviour commenced when the whole group of breeders started to form aggregations near the bottom of the tanks (Fig. 1, Table 1, Suppl. Video S1), being comparable to a "loose ball" and occasionally aggregations became tighter as the fish swam closer together; nonetheless, in the majority of the observations a "tight ball" of fish was not formed. Also, during this stage, males were observed to become slightly darker and occasionally males were rubbing and nudging (Fig. 1, Table 1) some females close to the genital pore. The change in colour of males in addition to differences in size between males and females was also used to identify males. Territorial dominance or aggression amongst fish of the same or different sex was not observed.

In parallel to the aggregation behaviour, fish (males and females) initiated the courtship behaviour, which was mostly brief, and started when one of the females increased swimming speed at the bottom of the tank and slightly separate from the rest of the group, although on repeated occasions this was punctuated by immobile periods of the female in mid-water column and periods of circling aggregation behaviour as described above. After 10-15 sec of this behaviour, the female with one or more males was observed to dramatically increase swimming speed to initiate the spawning rush (Fig. 1, Table 1). Aggregation and courtship behaviour ranged from 5 to 70 min to average 21 ± 4 min in both tanks.

— *Spawning rush.* The spawning rush was observed to follow when either i) the female started a circling behaviour followed by the male(s), which again produced the "loose ball" aggregation behaviour described previously, but the female would then exit from the group at speed or ii) after an apparently coincidental brief encounter with a male, the female dramatically increased swimming speed. This dramatic increase in swimming speed by the female was in all cases from low in the water column close to the bottom in a diagonal line towards the water surface. The female swam rapidly away from other fish followed by one or more males (Figs. 1 and 2; Table 1, Suppl. Video S1). Gamete release, egg and sperm, were synchronous at speed during the rush and in the top half of the water column.

The rush lasted in average 1.6 ± 0.5 sec in both tanks (Fig. 1). During this phase, the female was mostly

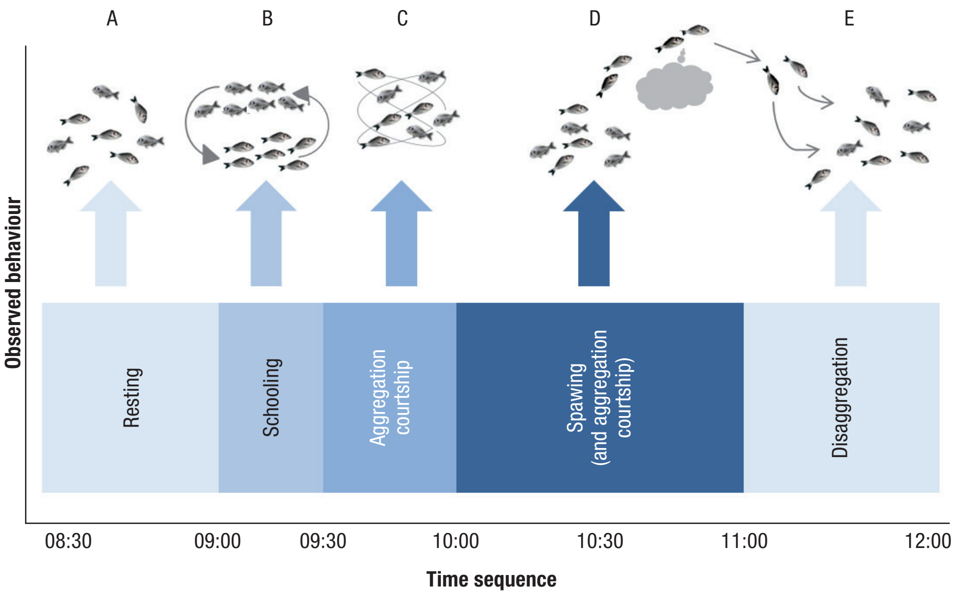

Figure 1. Average time periods of the different behavioural patterns observed in the gilthead seabream during the pre-spawning and the spawning events.

Table 1. Description of courtship and spawning behaviour for marine fish adapted for gilthead seabream (*Sparus aurata*) (modified from Erisman & Allen, 2006).

Behaviour	Description
Resting	Fish are disaggregated around the tank
Schooling	Breeders form groups, start to swim together with uniform movements
Rub-Nudge	Male approach gravid female and makes physical contact, with mouth, in the lower abdomen near the genital pore
Aggregation- courtship	Males swim near the females forming like a tight ball and made several contacts, this pattern commonly preceded the spawning rush of fish
Spawning rush	Female and a male separates from group and swim rapidly in a straight line while close together, male directly behind (at times touching) the female and oriented in the same manner. Rushes vary in direction from diagonally vertical (most common) to horizontal (rare); rush ends with synchronized gamete liberation, after which the fish separated

swimming in a head-down position exposing her abdomen to males, which were always positioned beneath her and with the lead male snout close to or touching the abdomen and oviduct area of the female (Fig. 2). In addition, males were observed to swim with open mouths at the moment of gametes liberation. Once gametes were released, the spawners separated and returned to swim around the tank to subsequently re-integrate into the group (Fig. 1, Table 1) until the initiation of another spawning event.

Spawning rushes involving more than one female were not observed in the present study. The presence of a second and a third male was observed on repeated occasions, although these second and third males were always behind the lead male during the spawning rush.

— *Disaggregation.* The spawning behaviour (from the formation of the aggregation to the end of the spawning rush) ended when the group of fish disaggregated and returned to a resting behaviour, with fish dispersed, swimming in all directions and biting the floor as if they were looking for food (Fig. 1). In addition all the fish in the group presented a similar colour and no dark males were observed, which also appeared to indicate the end of the spawning behaviour.

Spawning pattern in both broodstocks

— *Aggregations and courtship behaviour.* From the 67 spawning events observed, 35 corresponded to the

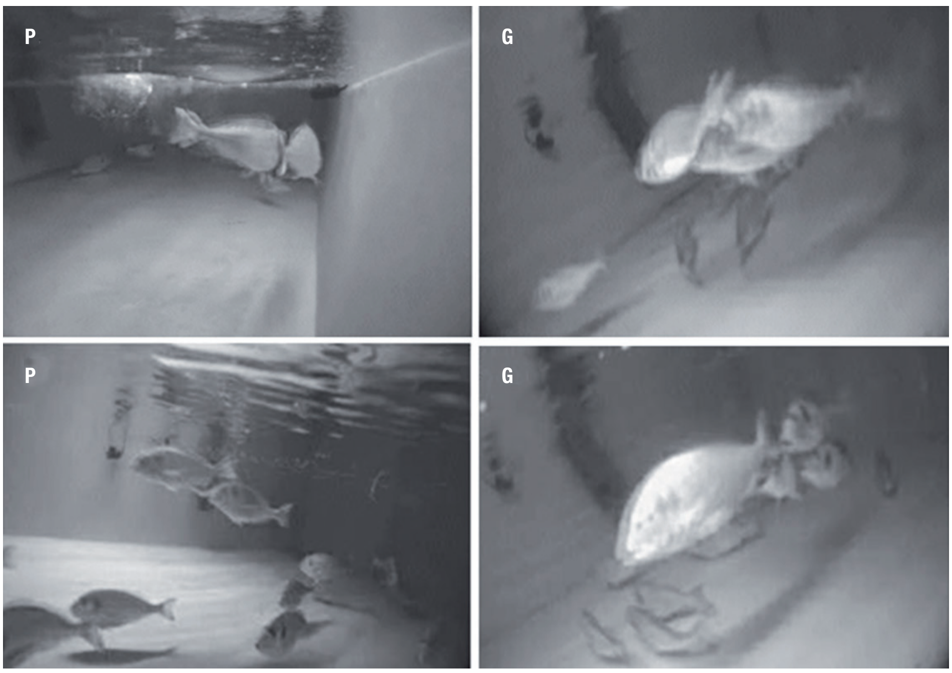

Figure 2. Video captures of the gilthead seabream spawning rush. P shows two examples of a pair spawning with the female followed by the male; G shows two examples of group spawning, the upper photo shows a female followed by two males and the lower photo a female followed by three males.

Table 2. Means values of different spawning patterns observed in the gilthead seabream.

Spawning parameter	Tank C1	Tank C2
Number of spawn with aggregations	25	13
Number of spawn without aggregations	10	19
Number of spawn with courtships	20	14
Number of spawn without courtships	14	19
Mean spawning activity per day (min)	50 ± 4	57 ± 5
Number of spawns per day	5.83 ± 0.21	5.33 ± 0.32
Mean distance displaced (m) per rush	1.8 ± 0.2	1.6 ± 0.3
Mean eggs volume (mL) spawned	343 ± 12	467 ± 15
Mean fertilisation rate (%)	95 ± 0.2	88 ± 0.5

breeders in tank C1 and 32 to the breeders in tank C2. The spawning rush initiated from a fish aggregation behaviour in 38 of the spawns (25 in tank C1 and 13 in tank C2,) and 29 events occurred without an aggregation immediately prior to spawning (10 corresponded to tank C1 and 19 in tank C2) (Table 2). Thirty four spawns occurred after the aggregation and courtship behaviour (described above, female increased swimming speed and momentarily froze) between a male and a female (20 in tank C1 and 14 in tank C2) and 33 occurred without this courtship behaviour immediately prior to the spawning rush (14 in tank C1 and 19 in tank C2). No significant differences were observed between the two broodstocks groups in the number of spawns recorded with aggregations and without aggregations or courtships (Table 2).

— *Spawning duration and frequency of spawns per day.* The duration and frequency of the spawning activity in the two broodstocks, kept in tank C1 and C2, were not significantly different. Fish spawning activity lasted in average 50 ± 4 and 57 ± 5 min in tank C1 and tank C2 (Table 2), respectively. The average numbers or frequency of spawns per day recorded were 5.83 ± 0.21 in tank C1 and 5.33 ± 0.32 in tank C2 (Table 2); and after completing the spawning rush breeders, from both tanks, returned back to the group.

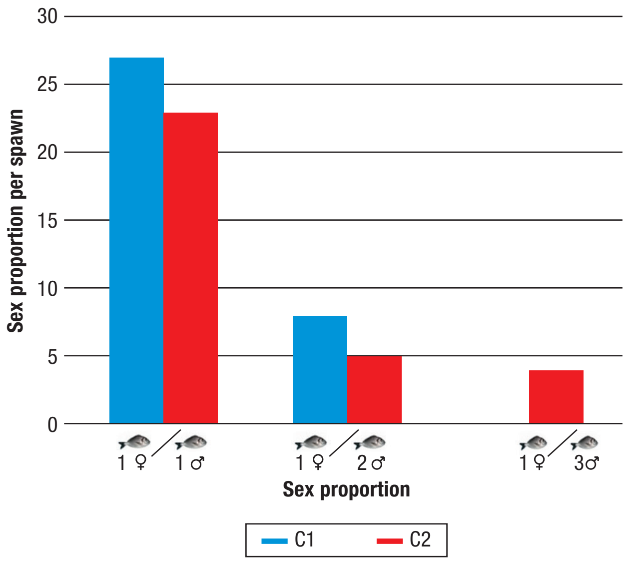

Figure 3. Sex proportion per spawning in the gilthead seabream (*Sparus aurata*) held in captivity.

— *Distance of spawning rush and preferred area to spawn.* The approximate distance displaced by broodstock in tank C1 (1.8 ± 0.2 m) to liberate the gametes during the spawning rush was no different from broodstock held in tank C2 (1.6 ± 0.3 m) (Table 2). Also, on the 67 recorded spawning events it was observed that seabream spawned 39.8% in the water inlet area of the tanks, 37.4% in the middle part and, finally, 22.7% spawns occurred in the water outlet area of the tanks. It was also observed that seabream spawned in the majority of occasions near the water surface.

— *Sex proportion per spawn.* All spawns were with the attendance of only one female with one or more males. A total of 50 pair spawns were recorded with one female and one male (27 corresponded to tank C1 and 23 in the tank C2). Group spawning was also observed, 13 spawning events were observed with one female and two males (8 in tank C1 and 5 in tank C2) and 4 spawns were with the presence of one female with three males (all 4 corresponded to broodstock held in tank C2) (Fig. 3). No significant differences were observed between the proportion of pair and group spawning in the two broodstocks. The spawning by two females at the same time was not observed, no spawns were observed that involved more than one female and two or more females were not observed to spawn at same time in different spawning rushes in different areas of the tank. Mass spawning, including all individuals spawning as a single unit, was not observed during the present study.

— *Eggs volume, fertilization rate and developmental stage.* Regular daily spawning began in both tanks in early January and spawning finished in June. The peak period of spawning in both tanks extended late January to mid-April and during the period 24 Jan to 15 April the mean volume of daily floating eggs were 434 ± 193 mL from tank C1 and 273 ± 155 mL from tank C2. On the days selected to analyse the spawning behaviour the breeders in tank C1 spawned a daily mean of 343 ± 12 mL and in tank C2 spawned 467 ± 15 mL (Table 2). No correlation was found between the number of spawning events per day and the volume of eggs collected ($R^2 = 0.2323$, $p > 0.221$). Eggs collected from tank C1 presented a fertilization rate of 95 ± 0.2%, while in tank C2 the mean fertilization rate was 88 ± 0.5% (Table 2). The embryonic phase of development of the collected eggs were mainly between 2 and 32 cell division (phases 1A to 1E as defined by Kamaci *et al*., 2005); nonetheless, on occasions it was observed some eggs to be in morula or gastrula phase (1F and 1G) and these developmental phases corresponded to the timing of the observed spawning events.

Discussion

The present study described, for the first time, the reproductive behaviour of gilthead seabream (*Sparus aurata*) held in captivity. The reproductive behaviour was similar to that described for other Sparidae species (Smith, 1986; Buxton, 1990; Buxton & Garratt, 1990; Garratt, 1991; Leu, 1994; Mylonas *et al*., 2011). Gilthead seabream were observed to form defined aggregations prior to the spawning event and females were observed to make a spawning rush with one or more males that finished with gamete liberation.

In accordance with Domeier & Colin (1997) the aggregation behaviour performed by fish was defined as a group of conspecific fish that gathered for the purpose of spawning, with fish densities or numbers significantly higher than those found in the area during the non reproductive period. In the present study, gilthead seabream aggregations were well defined, included the participation of all the stock and were clearly associated with spawning. The courtship behaviour of gilthead seabream was mostly brief and characterized by rapid forward swimming by females followed by one or more males. In addition, males displayed two characteristics: a colour change to become slightly darker and nudging and rubbing the female´s bellies close to the oviduct. The formation of aggregations and the courtship (changes in swimming speed, colour changes and nudging) appeared to offer the opportunity for mate selection and brought all the available individuals together for mate selection. Dichromatism (ability to take on one of two different colours patterns separately) was suggested to be a motivational factor for females to select males with better physical condition and social status (Kodric-Brown, 1998; Okumura *et al*., 2002; Kline *et al*., 2011). The action of rubbing and nudging was hypothesized to help males to perceive female pheromones, trigger the ovulation and induce the oocytes liberation (Bond, 1996; Domeier & Colin, 1997; Heyman *et al*., 2005; Stacey & Sorensen, 2008). In the present study, obvious behaviours associated with gaining dominance were not observed between males or males and females. However, a passive process of selection between males and females can be suggested as both observations of behavioural and morphological aspects appeared to offer opportunities for females to accept or reject advances from males. These indications that presented opportunities consisted of: a) spawning was often in a pair indicating the pair could select each other, b) aggregations brought all the fish together for close contact to aid selection and spawning was often soon after an aggregation, c) males followed females perhaps seeking selection, d) males nudged females to possibly stimulate selection, e) females were observed to swim away from advances from males and f) males changed colour changing appearance to perhaps aid selection by the female. Aggregations and/or courtship behaviours similar to the present study have been described in other species of Sparidae including silver seabream (*Chrysophrys auratus*) (Smith, 1986; Mylonas *et al*., 2011), santer seabream (*Cheimerius nufar*) (Buxton & Garratt, 1990; Garratt, 1991), roman seabream (*Chrysoblephus laticeps*) (Buxton, 1990), silver bream (*Rhabdosargus sarba*) (Leu, 1994) and southern black bream (*Acanthopagrus butcheri*) (Mylonas *et al*., 2011) and non-Sparidae such as the spotted sand bass (*Paralabrax maculatofasciatus*) (Miller & Allen, 2006), yellowtail amberjack (*Seriola lalandi*) (Moran *et al*., 2007), dusky grouper (*Epinephelus marginatus*) (Zabala *et al*., 1997), cubera snapper (*Lutjanus cyanopterus*) (Heyman *et al*., 2005) and white seabass (*Atractoscion nobilis*) (Aalbers & Drawbridge, 2008).

However, in the present study, aggregations were not always observed immediately prior to gilthead seabream spawning and no inter-individual dominances were observed. Liberation of gametes was observed both in gilthead seabream coming from an aggregation (with or without courtship) and fish that had not participated in aggregation (or courtship) behaviour immediately prior to spawning. However, the importance of these social interactions (aggregations and courtship) during the spawning period should not be lessened by these observations. Gilthead seabream spawning success was low when held in pairs (Gorshkov *et al*., 1997; N. Duncan, *pers. obs*.) or groups of 15 females with a single male (Gorshkov *et al*., 1997). Holding gilthead seabream in pairs or 15 females with a single male would be too few fish or the wrong sex ratios to enable the social interactions (aggregations and courtship) observed in the present study and this may explain the poor spawning success observed in gilthead seabream held in pairs or small groups (Gorshkov *et al*., 1997; Duncan *et al*., 2013). Various authors have suggested large groups of breeders were required for successful spawning of gilthead seabream (Gorshkov *et al*., 1997; Duncan *et al*., 2013) and Sparidae in general (Pankhurst, 1998; Mylonas *et al*., 2011).

In the present study, gilthead seabream made a spawning rush with a preference to rush and spawn as a pair and 71.6% of total spawns were observed to be between a single female and male. However, gilthead seabream were also observed to group spawn when one female spawned with several males: two (22.5%) or three males (4.9%). Species from the Sparidae family all presented a spawning rush and different species presented pair or group or both types of spawning. The silver seabream (Smith, 1986; Mylonas *et al*., 2011) and santer seabream (Buxton & Garratt, 1990; Garratt, 1991), like the gilthead seabream presented both pair and group spawning. However, silver seabream were predominantly group spawners with one female being followed by many males (Smith, 1986; Mylonas *et al*., 2011), but pair spawning was observed on one occasion (Smith, 1986). Santer seabream pair spawned (Buxton & Garratt, 1990; Garratt, 1991) and the dominant male was aggressive towards other males, however, on occasions a "streaker" or "sneaker" male was observed to successfully participate in spawns by keeping to the opposite side of the female to the dominate male (Garratt, 1991). In the present study, no evidence of sneaker males was observed in gilthead seabream, although, when group spawning was observed there was always a lead male closest to the female followed by a second and less frequently a third male. The roman seabream (Buxton, 1990) and silver bream (Leu, 1994) were only observed to pair spawn and the southern black bream was only observed to group spawn (Mylonas *et al*., 2011). To date no Sparidae species has been observed to mass spawn and the observed pair and/or group spawning preceded by social interactions related to mate selection were characteristic of gilthead seabream and other Sparidae species.

Domeier & Colin (1997) defined a mass spawning as "*a form of group spawning that consists of the great majority to all of an aggregation spawning simultaneously, as a single unit*". Studies on parental assignment of progeny (Brown *et al*., 2005; Chavanne *et al*., 2012) have referred to gilthead seabream spawning behaviour as mass spawning. However, the present study found that gilthead seabream only participated in pair and group spawning in agreement with other studies on Sparidae species. Nevertheless, all these observations were made on fish held in captivity and no reports have been published on the reproductive behaviour of wild populations of Sparidae. Although to date no study on a Sparidae species has observed mass spawning this spawning type cannot be discounted as a possible spawning behaviour in Sparidae and gilthead seabream. Mass spawning reproductive behaviour has been documented in several marine fish species such as the *Lutjanus cyanopterus* (Heyman *et al*., 2005) and the *Dermatolepis dermatolepis* (Erisman *et al*., 2009). Both species were observed in natural conditions and fish were described to release a massive cloud of gametes into the water column that made observation difficult. Females were, however, observed to exit from the mass spawning aggregations with accompanying males in examples of simultaneous group spawning. Therefore, the group spawning observed in Sparidae and the gilthead seabream could form part of mass spawning in different conditions. Domeier & Colin (1997) in an extensive review of aggregations and spawning type observed that species change spawning type in relation to the situation, with pair spawning more common in the absence of an aggregation and group spawning more common in aggregations and mass spawning was observed in some species to involve many incidents of simultaneous group spawning (as mentioned above). However, caution should be used in referring to a species such as gilthead seabream as mass spawning when only pair and group spawning has been observed.

Parental assignment of progeny also identified that the participation of gilthead seabream breeders was variable with a proportion of breeders that did not participate in spawning (Brown *et al*., 2005; Porta *et al*., 2009; Chavanne *et al*., 2012) and this variation or dominance by certain fish was particularly clear amongst male breeders (Brown *et al*., 2005). A similar situation was observed in the parental assignment of male cod breeders to progeny (Bekkevold *et al*., 2002) and this coupled with observations of cod reproductive behaviour (Brawn, 1961; Hutchings *et al*., 1999) suggested that cod males had reproductive hierarchies that explained the dominance of progeny by certain males (Bekkevold *et al*., 2002). A similar coupling of the present study on gilthead seabream spawning behaviour with studies on parental assignment of gilthead seabream progeny (Brown *et al*., 2005; Porta *et al*., 2009; Chavanne *et al*., 2012) also suggested the hypothesis that gilthead seabream had reproductive hierarchies that resulted in the dominance of progeny by certain breeders particularly amongst males. Chavanne *et al*. (2012) concluded that further research was required to understand the spawning kinetics of gilthead seabream. The present study, highlights that such studies need to also focus on spawning behaviour to understand why certain fish dominate spawning in relation to the spawning environment considering both physical (tank design, size) and social (characteristics of individuals, sex ratios, density) aspects. This, the first description of gilthead seabream spawning behaviour provides an important bases for these studies and for the first time researchers and broodstock managers can have a clear idea of the spawning behaviour when considering physical and social manipulations to increase parental contribution for breeding programs.

In the present study, the spawning activity took place midmorning, which was actually initiated 42 ± 8 min after the lights switched on and can be considered similar to previous studies that established that gilthead seabream and others sparid fish such as silver seabream (*Sparus sarba*), Pacific seabream (*Acanthopagrus pacificus*), yellowfin bream (*Acanthopagrus australis*), red seabream (*Pagrus major*) and black bream (*Acanthopagrus butcheri*) tend to spawn at sunset or early in the morning (Pollock, 1982; Matsuyama *et al*., 1988; Mihelakakis & Kitajima, 1995; Haddy & Pankhurst, 1998; Meseguer *et al*., 2008; Sheaves & Molony, 2013). In the present study, spawning was successfully and regularly obtained and presented a prolonged spawning season (up to 5 months). Spawning was close to every day in both tanks. These observations were characteristic of this species, and in accordance with Zohar *et al*. (1995), Barbaro *et al*. (1997) and Arabaci *et al*. (2010).

The present study demonstrated that gilthead seabream spawning behaviour was similar to other sparids. In most occasions, spawns were observed to initiate in the morning hours and presented the characteristic to be associated with aggregation behaviour, followed by the spawning rush performed by a single female pursued by a male or, less common, by two or three males. Aggregation and courtship behaviour appeared to be an essential part of the spawning behaviour probably related to mate selection, highlighting the need to have a group of breeders and not single pairs. These findings described for the first time the characteristics of gilthead seabream reproductive behaviour and that many spawning events during a short space of time were involved in the production of a “spawn”. Altogether the study provided valuable information that may explain the uneven participation of breeders in studies that determined paternity of progeny with microsatellites and provides a solid basis for future work to increase parental contributions to breeding programs.

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